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Human carriage of cefotaxime resistant *Escherichia coli* in North-East India: an analysis of sequence types and associated resistance mechanisms.

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Short running title : *E. coli* ST in North East India

1 SYNOPSIS

2 **Objectives:** To determine the prevalence of *E. coli* sequence types and associated resistance
3 mechanisms carried by the community in North-Eastern India.

4 **Methods:** *E. coli* (108) were isolated from sewage collected from 19 sites across the city of
5 Silchar by plating on MacConkey agar with/without selection (50mg/L **ceftotaxime**). Species
6 identification was confirmed by MALDI-TOF MS for **82 isolates**. Common resistance
7 mechanisms were determined by WGS of pooled *E. coli* isolates. PFGE combined with specific
8 probes determined the presence of common resistance mechanisms in all isolates. Phylotype,
9 MLST, cgMLST, resistance gene and virulence gene content were determined by in silico
10 analysis of 38 genomes.

11 **Results and conclusions:** Analysis of isolates collected without selection (n=33) indicated that
12 CTX resistance in *E. coli* was 42% (14/33) and estimated meropenem resistance at 9%. The
13 remaining 58% (19/33) were additionally sensitive to ampicillin, trimethoprim, ciprofloxacin
14 and aminoglycosides. The most common ST among the CTX resistant *E. coli*'s was ST167 (29%)
15 followed by ST410 (17%) and ST648 (10%). *E. coli* ST131 was absent from the collection.

16 **Sixty-three isolates were resistant to cefotaxime, and harboured *bla*_{CTX-M-15}, 54% (34/63) or**
17 ***bla*_{CMY-42}, 46% (29/63) of which 10% (6/63) harbored both genes. Carbapenem resistance was**
18 **due to *bla*_{NDM-5}, found in 10/63 CTX resistant isolates and/or *bla*_{OXA-181} found in 4/63 isolates.**
19 **NDM-5 was encoded by IncX3 and/or IncFII plasmids and CMY-42 was mostly encoded by IncI**
20 **plasmids. NDM-5 appears to have replaced NDM-1 in this region and CMY-42 appears to be in**
21 **the process of replacing CTX-M-15.**

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26 INTRODUCTION

27 *Escherichia coli* is universally carried in the human gut and is one of the most common
28 bacterial pathogens causing a range of disease manifestations.¹ Importantly, the main source of
29 infection is the patients' own digestive tract with the main site of infection the urinary tract.
30 *E. coli* is the leading cause of urinary tract infections² and subsequent septicaemia's in many
31 nations.^{3,4} *E. coli* has an "open genome" meaning that as a species it easily gains and loses
32 genetic information.⁵ Thus comparison of *E. coli* genomes has revealed that the core genome
33 only consists of c. 1000 genes (1/5th of the genome of each isolate).⁶ This diverse genetic make-
34 up means that any individual isolate may be a serious pathogen or an innocuous commensal
35 and therefore typing of common community carriage isolates is useful to understand the link
36 between infection and prevalence of gut carriage of virulent and/or antibiotic resistant clones.
37 Antibiotic resistance is also an important marker since increasing antibiotic resistance
38 prevalence is associated with both increasing bacteraemia rates³ and associated mortality due
39 to delay in appropriate therapy.⁷ Furthermore, rise in the prevalence of gut carriage of a
40 virulent and antibiotic resistant strain of *E. coli* has a compounded effect.³ This is important
41 since European bacteraemia rates have substantially increased over the last 20 years, partially
42 due to rising carriage rates of a virulent and often multi-drug resistant *E. coli* strain, ST131.³
43 We have previously shown that common *E. coli* sequence types and resistance mechanisms can
44 vary dramatically in different human communities.⁸ To further understand this observation we
45 sought to determine these in the city of Silchar, North East India.

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51 **Materials and Methods:**

52 **Collection of samples**

53 Human sewage samples (30mL) were collected in January 2018 from 19 sites across the city of
54 Silchar, Assam, India.

55 **Bacterial Identification**

56 Bacteria were pelleted by centrifugation and resuspended in 500µL LB broth. 50µL of several
57 serial dilutions were then spread on MacConkey agar plates with and without 50mg/L
58 cefotaxime. Up to 10 colonies with typical *E. coli* morphology were randomly collected from
59 each site, 5 with and 5 without selection (55 and 53 with and without selection, respectively)
60 of which 49 and 33 were confirmed as *E. coli* by MALDI-TOF MS.

61 **Genomic DNA extraction**

62 Genomic DNA was extracted using the Qiagen genomic DNA kit.

63 **MiSeq sequencing**

64 DNA libraries were prepared using the Nextera XT sample kit and sequenced at 30X coverage
65 with a standard 2X 100 base protocol on a MiSeq instrument (illumina, San Diego, CA, USA).

66 **Initial WGS analysis**

67 Isolates were pooled into ten pools consisting of 8 isolates per pool and sequenced at 30X
68 coverage by illumina Miseq to give an indication of the range of resistance mechanisms in the
69 samples.

70 **PFGE and specific probing**

71 PFGE was performed as described previously.⁹ Gels were probed directly using radio-labeled
72 probes for *bla*_{NDM}, *bla*_{CTX-M-15}, *bla*_{CMY-42}, *qnrS1*.

73 **ST and virulence gene detection**

74 The MLST of sequenced strains was determined with StringMLST using short read data in
75 FASTQ format and Ridom Seqsphere + (version 3.5.0) using assembled data in FASTA format.

76 CH typing (*fumC/fimH*) was used to indicate the MLST group of non-sequenced strains.¹⁰ *E. coli*
77 strains were clustered based on core genome MLST (cgMLST). Antimicrobial resistance genes
78 were detected using CLC Biogenomic workbench. CH types, plasmid and virulence genes were
79 determined online using CH typer, Plasmid finder and Virulence finder
80 (<http://www.genomicepidemiology.org/>).

81 Phylogroup analysis

82 The *E. coli* phylotypes were determined with in-silico searches for *chuA*, *yjaA*, *tspE4C2*, *arpAgpE*
83 and *tnpAgpC* using geneious software based on the Clermont method.^{11, 12}

84

85 Results and discussion

86 We isolated 82 *E. coli* collected from 19 sites across the city of Silchar to determine the *E. coli*
87 ST and associated resistance mechanisms carried by the local population. The 82 *E. coli*
88 included 33 isolates collected without selection and 49 by selection on 50mg/L cefotaxime.
89 Fifty-eight percent of isolates collected without selection (19/33) were cefotaxime susceptible
90 and also susceptible to all tested antimicrobials including ciprofloxacin, trimethoprim and
91 gentamicin. Sixty-three isolates were cefotaxime resistant including 14/33 collected without
92 selection, thus 42% of carriage *E. coli* in Silchar are cefotaxime resistant. Twenty-percent
93 (10/49) of cefotaxime resistant isolates were also resistant to carbapenems giving an estimate
94 of carriage of carbapenem resistance in *E. coli* of 9% (42/100 X 10/49). Cefotaxime and
95 carbapenem resistant isolates were found at 100% and 47% (9/19) of sample sites,
96 respectively (Figure 1, Table S1). The total complement of resistance mechanisms was initially
97 determined by MiSeq sequencing of ten pools of eight isolates at 30X coverage. This indicated a
98 high prevalence of resistance genes *bla*_{CTX-M-15}, *bla*_{CMY-42}, *bla*_{NDM-5} and *qnrS1* and allowed us to
99 target their presence by PCR/sequencing and genomic location by in-gel radio-labeled probing
100 of PFGE gels (Table S1). The most common gene conferring cefotaxime resistance was *bla*_{CTX-M-}

15 found in 34/63 cefotaxime resistant isolates followed by *bla*_{CMY-42} (29 isolates) and *bla*_{NDM-5} (10 isolates). One isolate contained all three β -lactamases and six isolates contained both *bla*_{CTX-M-15} and *bla*_{CMY-42} genes, several isolates contained multiple β -lactamase genes (Figure 1, Table S1). The *bla*_{NDM-5} and *bla*_{CMY-42} genes were found on plasmids ranging in size from 85-140kb and 25-130kb (Table S1), respectively. However, *bla*_{CTX-M-15} and *qnrS1* genes were found mostly on the chromosome 66% (22/33) or on plasmids of 40-175kb. 38 individual isolates were further chosen for whole genome sequencing to determine the MLST types and resistance and virulence gene complements (Figure 1, Figure S1, Table S1). In addition, a further 10 isolates were typed by the Weissman 2 locus scheme. MLST was determined for 48 isolates (Figure 1, Figure S1, Table S1), the most prevalent being ST167, 29% (14/48), followed by ST410 17% (8/48), ST648 10% (5/48), ST224 (2/48), ST609 (2/48), ST973 (2/48), ST2083 (2/48) and single isolates of ST46, ST84, ST101, ST156, ST215, ST315, ST361, ST617, ST405, ST2521, ST3268 and ST4450 and one new ST (Figure 1, Table S1). ST167 was found at 9/19 sites and ST410 was found at 6/19 sites. Over half of all of isolates (56%) belonged to ST167, ST410 and ST648 (Figure 1). In a recent UK nationwide study of cefotaxime resistant *E. coli* isolated from human faeces (360 isolates), sewage (65 isolates) and bacteraemia's (293) ^{13, 14} the most prevalent *E. coli* were ST131 and ST38 (44%, 31% and 70% of all isolates from faeces, sewage and bacteraemia's, respectively). Interestingly, in this study both ST38 and ST131 were absent. Thus, the prevalence of *E. coli* ST carriage varies greatly by geographic location. In Silchar, the common sequence type ST167 was closely associated with *bla*_{NDM-5}, 50% (7/14) and both ST167 and ST410 with *bla*_{CMY-42}, 65% (9/14) and 100% (8/8), respectively (Figure 1). This high prevalence of *bla*_{CMY-42} has not been documented before in India or elsewhere and appears to be in the process of replacing *bla*_{CTX-M-15} at this location. This may be related to the wider spectrum of β -lactam hydrolysis of CMY-42 as compared to CTX-M-15. Similarly *bla*_{NDM-5} appears to have replaced *bla*_{NDM-1} in Silchar as no isolates were detected with *bla*_{NDM-1}. Whilst

126 this may be a local **phenomenon**, it is interesting that many nations have reported increased
127 detection of *bla*_{NDM-5} over the last few years.^{15, 16, 17, 18} Many isolates harboured other
128 resistance mechanisms (Figure S1, Table S1) and chromosomal mutations conferring high level
129 fluoroquinolone resistance making many strains MDR (Figure S1, Table S1). However,
130 fosfomycin and chloramphenicol resistance was low (only 3 strains produced a full-length
131 chloramphenicol resistance gene) suggesting that these antibiotics could be useful treatment
132 options in this locale. Phylotype and virulence gene analysis indicated that most isolates
133 belonged to non-pathogenic phylogroups A and B1, which included the majority of isolates
134 carrying carbapenemase genes (ST167 and ST410). Six isolates belonged to the pathogenic
135 phylotype D (ST268, ST315, STNEW, ST405, 2X ST 973, Figure S1) yet none belonged to the B2
136 group. Many of the isolates (20/48) including 2x ST648 and the majority of ST167 isolates
137 were missing the type 1 fimbrial adhesion *fimH* which has been shown to be essential for
138 colonizing the urinary tract. The cgMLST (Figure S1, Figure 2) agreed with the phylogroup
139 analysis and demonstrated that all sequenced strains were unique confirming the random
140 nature of the selection process. The within ST SNP analysis further demonstrated this and
141 highlighted that differences within ST648 and ST167 were more numerous than within ST410,
142 perhaps suggesting that the ST410 have expanded within the Silchar population in more recent
143 history.

144 In conclusion, our survey has revealed that the majority of *E. coli* strains in this area are fully
145 sensitive to antibiotics. However, 42% and 9% of isolates are cefotaxime and carbapenem
146 resistant, respectively. We also found that the majority of resistant strains belonged to just
147 three prevalent ST, which are different to the prevalent *E. coli* ST in Europe. Notably, *E. coli*
148 ST131, the dominant **cefotaxime** resistant strain found throughout Europe and North America
149 was absent from our study. Since UTI infections typically originate in the community and from

150 the human gut, the survey of resistant human carriage isolates is a useful surveillance
151 approach to quickly identify common ST and resistance mechanisms and guide local therapy.

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157 **Transparency declaration**

158 All authors have none to declare.

159

160 **References**

- 161 1. Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to
162 *Escherichia coli*: focus on an increasingly important endemic problem. *Microbes Infect*
163 2003; **5**: 449-56.
- 164 2. Schito GC, Naber KG, Botto H *et al*. The ARES study: an international survey on the
165 antimicrobial resistance of pathogens involved in uncomplicated urinary tract
166 infections. *Int J Antimicrob Agents* 2009; **34**: 407-13.
- 167 3. de Kraker ME, Jarlier V, Monen JC *et al*. The changing epidemiology of bacteraemias in
168 Europe: trends from the European Antimicrobial Resistance Surveillance System. *Clin*
169 *Microbiol Infect* 2013; **19**: 860-8.
- 170 4. Laupland KB. Incidence of bloodstream infection: a review of population-based studies.
171 *Clin Microbiol Infect* 2013; **19**: 492-500.
- 172 5. Lukjancenko O, Wassenaar TM, Ussery DW. Comparison of 61 sequenced *Escherichia*
173 *coli* genomes. *Microb Ecol* 2010; **60**: 708-20.

- 174 6. Land M, Hauser L, Jun SR *et al.* Insights from 20 years of bacterial genome sequencing.
175 *Funct Integr Genomics* 2015; **15**: 141-61.
- 176 7. Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with
177 extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a
178 systematic review and meta-analysis. *J Antimicrob Chemother* 2007; **60**: 913-20.
- 179 8. Zahra R, Javeed S, Malala B *et al.* Analysis of *Escherichia coli* STs and resistance
180 mechanisms in sewage from Islamabad, Pakistan indicates a difference in *E. coli*
181 carriage types between South Asia and Europe. *J Antimicrob Chemother* 2018.
- 182 9. Toleman MA. Direct in Gel Genomic Detection of Antibiotic Resistance Genes in S1
183 Pulsed Field Electrophoresis Gels. *Methods Mol Biol* 2018; **1736**: 129-36.
- 184 10. Weissman SJ, Johnson JR, Tchesnokova V *et al.* High-resolution two-locus clonal typing
185 of extraintestinal pathogenic *Escherichia coli*. *Appl Environ Microbiol* 2012; **78**: 1353-60.
- 186 11. Clermont O, Christenson JK, Denamur E *et al.* The Clermont *Escherichia coli* phylo-typing
187 method revisited: improvement of specificity and detection of new phylo-groups.
188 *Environ Microbiol Rep* 2013; **5**: 58-65.
- 189 12. Beghain J, Bridier-Nahmias A, Le Nagard H *et al.* ClermonTyping: an easy-to-use and
190 accurate in silico method for *Escherichia* genus strain phylotyping. *Microb Genom* 2018;
191 **4**.
- 192 13. Livermore DM, Day M, Cleary P *et al.* OXA-1 beta-lactamase and non-susceptibility to
193 penicillin/beta-lactamase inhibitor combinations among ESBL-producing *Escherichia*
194 *coli*. *J Antimicrob Chemother* 2019; **74**: 326-33.
- 195 14. Sattar H, Toleman M, Nahid F *et al.* Co-existence of bla(NDM-1) and bla(KPC-2) in
196 clinical isolates of *Klebsiella pneumoniae* from Pakistan. *Journal of Chemotherapy* 2016;
197 **28**: 346-9.

15. Li X, Fu Y, Shen M *et al.* Dissemination of blaNDM-5 gene via an IncX3-type plasmid among non-clonal *Escherichia coli* in China. *Antimicrob Resist Infect Control* 2018; **7**: 59.
16. Huang Y, Yu X, Xie M *et al.* Widespread Dissemination of Carbapenem-Resistant *Escherichia coli* Sequence Type 167 Strains Harboring blaNDM-5 in Clinical Settings in China. *Antimicrob Agents Chemother* 2016; **60**: 4364-8.
17. Howard JC, Creighton J, Heffernan H *et al.* Evidence of transmission of an NDM-5-producing *Klebsiella pneumoniae* in a healthcare facility in New Zealand. *J Antimicrob Chemother* 2017; **72**: 949-51.
18. Reynolds ME, Phan HTT, George S *et al.* Occurrence and characterization of *Escherichia coli* ST410 co-harbours blaNDM-5, blaCMY-42 and blaTEM-190 in a dog from the UK. *J Antimicrob Chemother* 2019.

Legend Figure 1

This figure appears in colour in the online version of JAC and in Black and white in the printed version of JAC.

The association of β -lactamase resistance mechanism with *E. coli* ST for 48 carriage isolates. The Inner ring corresponds to the percentage of cefotaxime resistant *E. coli* belonging to each sequence type. MLST was determined in silico for 38 isolates that had been whole genome sequenced (Strain ID and isolation site in bold) and a further ten isolates by CH typing (normal font). The outer ring indicates the β -lactamase resistance mechanisms associated with each isolate. Isolate ID and isolation site is indicated outside the second ring. Isolates coloured yellow and green belong to commensal phylotype A. Isolates coloured white-blue belong to phylotype B1 and isolates coloured orange to red belong to pathogenic phylogroups F and D.

Legend figure S1

222 This figure appears in colour in the online version of JAC and in Black and hite in the printed
223 version of JAC.

224 Core genome analysis of sequenced isolates (38) with detail of non- β -lactam resistance
225 mechanisms. Chromosomal mutations in gyrase A and parC genes rthat result in amino acid
226 substitutions known to confer ciprofloxacin resistance in *E. coli* are given in the quinolone
227 column along with acquired genes that also confer quinolone resistance. Virulence genes and
228 *fimH* types as well as phylogroup are given for each strain. Isolates coloured yellow and green
229 belong to commensal phylotype A. Isolates coloured white-blue belong to phylotype B1 and
230 isolates coloured orange to red belong to pathogenic phylogroups F and D.

231 **Legend figure 2**

232 This figure appears in colour in the online version of JAC and in Black and hite in the printed
233 version of JAC.

234 SNP variation found among and between sequence types. Isolates belonging to the same
235 sequence type are highlighted as clusters 1-6. The number of SNP's found between members of
236 the same sequence types and between sequence types are given in bold adjacent to each isolate
237 pair. Cluster 2 ST410 isolates shared the least within ST SNP variation. Colours represent
238 different ST groups as in other figures.

239 **Table S1**

240 Table gives ST and CH data of all 63 cefotaxime resistant *E. coli* isolates collected in this study
241 together with entire resistance gene complements and plasmid size, incompatibility group and
242 genomic location of the most prevalent resistance genes. Whole genome sequenced isolates are
243 highlighted in bold. Isolates with *bla*_{CMY} genes other than *bla*_{CMY-42} are highlighted with an
244 asterix in the table ie DJ-95 produces *bla*_{CMY-145} and isolate DJ58 produces *bla*_{CMY-4}. The genomic
245 location of resistance genes is highlighted by plasmid size and chromosomal location of CTX-M-

246 15 genes by c. Selection or non selection is indicated by CTX (50mg/L cefotaxime) or NS,
247 respectively.

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249